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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Jakobsen et al.

Serial No.: 09/334,969

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For: Multivalent T-cell Receptor

Examiner: DiBrino, M.

Group Art Unit: 1644

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Nancy C. Gilmore

Nancy E. Gilmore

United States Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

AMENDMENT AND RESPONSE

This Amendment and Response is filed in reply to the Office Action dated January 17, 2001. A response to the Office Action was first due on April 17, 2001. A petition for a three-month Extension of Time for Response, up to and including July 17, 2001 is filed herewith. The Commissioner is authorized to debit the $890.00 fee for the Extension of Time, as well as any other fee necessary to maintain the pendency of this application, from Deposit Account No. 08-0219.

AMENDMENT

In the specification:

Pursuant to 37 CFR 1.121(b)(3) and 1.125(b), please replace the present specification with the substitute specification submitted herewith. A marked-up copy of the substitute specification, showing changes from the previous version is also submitted herewith.

In the claims:

Please amend claims 1, 7, 8, 10, 11, 14, 15, 22, 23, and 24 as noted on the attached sheet of paper, and cancel claims 12 and 13. Please add claims 33, 34, and 35. Please cancel claims 28-30 and 32, which were drawn to non-elected subject matter, without prejudice to further prosecution in a related application. As required by 37 C.F.R. §1.121(c), the amended claims are rewritten with all changes included. In addition, as permitted under 37 C.F.R. §1.121(c)(3), a clean version of all the pending claims is submitted as a single amendment paper, which is attached to this response. Also attached is the marked-up copy of the claims, marked to show all of the changes relative to the previous version of the claims. As permitted under 37 C.F.R. 1.121(c), this marked-up version does not include the cancelled claims.

RESPONSE

Claims 1-27, 28-30, and 32 were pending in the application. Claims 28-30 and 32 were withdrawn from consideration as being drawn to non-elected subject matter, and claims 1-27 were rejected. Claims 12, 13, 28-30, and 32 are cancelled herein, and claims 1, 7, 8, 10, 11, 14, 15, 22, 23, and 24 are amended. Claims 33-35 are added. Support for claims 33-35 can be found in claims 14-16 and throughout the specification. The specification is amended to correct informalities relating to the use of trademarks. Applicants respectfully submit that no new matter is introduced by any of these

amendments and request reconsideration of the application and amended claims in light of the following remarks. Claims 1-11, 14-27, and 33-35 are currently pending.

Restriction Requirement

The Office Action acknowledges Applicants' election without traverse of claims 1-27. Applicants have cancelled the non-elected claims, claims 28-30 and 32, by amendment herein.

Objections to the Specification

The specification was objected to as improperly using a number of trademarks. The uses of these trademarks have been corrected by amendment herein. In each case, the trademarks are now capitalized and followed by the ™ symbol. Applicants submit that the trademarked products were already accompanied by sufficient generic terminology in the originally filed specification and, therefore, no further amendments are necessary to describe the intended products.

Rejections under 37 C.F.R.§1.75(c)

Claims 14 and 15 were objected to under 37 C.F.R. §1.75© as being in improper form because a multiple dependent claim cannot depend from any other multiple dependent claim. Claim 14 has therefore been amended to depend from claim 1. Claim 15 depends from claim 14. In light of these amendments, Applicants respectfully submit that the objection under 37 C.F.R. §1.75(c) should be withdrawn.

Rejections under 35 U.S.C. §112, Second Paragraph

Claims 7-16, 22, and 24 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite. Specifically, the Office Action states that: (A) the use of "such as" in claims 7 and 8 is indefinite; (B) the use of the term "particle" in claim 24 is indefinite; (C) the term "multimerised" recited in claims 10 and 11, the terms "dimerisation," "heterodimerised," and "heterodimerisation" in claims 12, 14, 15, and 16, the term "disulphide" and the term "derivatised" in claim 22 should be changed to

"multimerized," "dimerization," " heterodimerized," "heterodimerization," "disulfide," and "derivatized," respectively; and (D) the recitation of the phrase "a first heterologous C-terminal dimerization peptide" in claim 12 is indefinite because it is unclear to what said peptide is heterologous.

With respect to rejection (A), Applicants respectfully acknowledge that the phrase "such as" in claim 7 is not standard U.S. patent practice and have amended the claim, deleting the entire phrase "such as avidin, streptavidin, or extravidin." Applicants note that the specification teaches that multivalent attachment molecules in general may be used to form multimers of TCR, and suggests that examples of multivalent attachment molecules include avidin, streptavidin, and extravidin (page 14, lines 24-29 to page 15, lines 1-5). The specification further teaches that other molecules which bind biotin in a multivalent manner would be suitable, and that multimers could also be formed by incorporating entirely different linkers, such as polyhistidine tracts bound to chelated nickel ion (page 24, lines 19-22). Thus, Applicants submit that there is support for multivalent attachment molecules generally.

Also with respect to rejection (A), Applicants again acknowledge that the phrase "such as" in claim 8 is not standard U.S. patent practice and have amended the claim to remove the phrase "a modifying enzyme such as biotin," and insert the phrase "an amino acid sequence encoding a protein tag." Applicants note that the specification teaches that a protein tag may comprise a sequence which encodes the recognition site for a modifying enzyme, and suggests that such an enzyme is the bacterial modifying enzyme BirA, which catalyzes biotinylation of lysine residues in its recognition sequence (page 7, lines 1-7). Protein tag sequences are not limited to those encoding modifying enzymes. Short tag sequences which can aid in the purification of the recombinant TCR α and β chains could also be included (page 21, lines 15-18). Thus, Applicants submit that there is support for the introduction of protein tag sequences generally.

With respect to rejection (B), Applicants have amended the claim to recite "solid structure." Applicants note that this change is supported by the specification at page 15, lines 14-16, which states, "suitable structures for use in the invention include membrane structures such as liposomes and solid structures which are preferably particles such as beads, for example latex beads." Applicants submit, therefore, that this amendment does not introduce any new matter. In light of the amendment, Applicants request that the rejection of claim 24 under 35 U.S.C. §112, second paragraph, be withdrawn.

With respect to rejection (C), Applicants respectfully submit that the spellings to which the Examiner objects are not incorrect, but, rather, are the British spellings of these words. Nonetheless, Applicants have amended the claims according to the Examiner's suggestions. In light of the amendments, Applicants respectfully request that the rejection of the claims on this ground be reconsidered and withdrawn.

With respect to rejection (D), Applicants believe one of ordinary skill in the art would understand, based upon a reading of the entire specification as a whole, that the term "heterologous" in claim 12 is intended to mean that the C-terminal dimerization peptide is heterologous with respect to the α or γ TCR chains. Nonetheless, claims 1 and 11, which have been amended to incorporate the limitations of canceled claim 12, recite a "first C-terminal dimerization peptide which is heterologous to the α or γ chain" (emphasis added). In light of the amendment, Applicants respectfully submit that claims 1 and 11 are in condition for allowance under 35 U.S.C. §112, second paragraph.

Rejections under 35 U.S.C. §102(b) over WO 97/35991

Claims 1-9, 10-12, 17, 26, and 27 were rejected under 35 U.S.C. §102(b) as being anticipated by WO 97/35991. Applicants note, however, that claim 13 was not rejected under U.S.C. §102(b) in view of WO 97/35991, as WO 97/35991 does not teach a multivalent TCR comprising recombinant TCR lacking the disulphide bond present between chains in native TCRs.

Applicants have amended claims 1 and 11 to incorporate a limitation corresponding to the limitation of cancelled claim 13. Claims 2-10, 17 and 26, and 27 depend directly or indirectly from claim 1, and claim 12 is cancelled herein. Therefore, in view of these amendments, Applicants submit that the rejections of claims 1-9, 10, 11, 17, 26, and 27 based upon WO97/35991 are overcome and should be withdrawn.

Rejections under 35 U.S.C. §102(b) over Paliwal et al.

Claims 1-4 and 17 were rejected under 35 U.S.C. 102(b) as being unpatentable over Paliwal et al. (1997), *J. Immunology* 159: 1718-1727 ("Paliwal et al."). Applicants note, however, that claim 13 was not rejected under U.S.C. §102(b) in view of Paliwal et al., as Paliwal et al. does not teach a multivalent TCR comprising recombinant TCR lacking the disulphide bond present between chains in native TCRs. In fact, Paliwal et al. specifically teaches a disulphide-linked α/β heterodimer, while the limitation of claim 13 is drawn to an α/β TCR heterodimer lacking the interchain disulphide bond found in the native TCR.

Applicants have amended claim 1 to incorporate a limitation corresponding to the limitation of cancelled claim 13. Claims 2-4 and 17 depend directly or indirectly from claim 1. Therefore, in view of these amendments, Applicants submit that the rejections of claims 1-4 and 17 based upon Paliwal et al. are overcome and should be withdrawn.

Rejections under 35 U.S.C. 103(a) over WO 97/35991 in view of Golden et al., and O'Shea et al.

Claims 1-9, 10-12, 14-18, 26 and 27 were rejected under 35 U.S.C. §103(a) as being unpatentable over WO 97/35991 in view of Golden et al.(1997), *J. Immunol. Meth.* 206: 163-169 ("Golden et al.") and O'Shea et al. (1989), *Science* 245: 646-648 ("O'Shea et al."). Applicants note that claim 13 was not rejected under 35 U.S.C. 103(a) in view of WO 97/35991, Golden et al., and O'Shea et al., as these references do not teach a recombinant TCR lacking the disulphide bond present between chains in native TCRs.

Because Applicants have amended claims 1 and 11 to incorporate a limitation corresponding to the limitation of cancelled claim 13, and because claims 2-10, 14-18, 26, and 27 depend directly or indirectly from claims 1 or 11, Applicants submit that the rejections of claims 1-9, 10-11, 14-18, 26 and 27 under 35 U.S.C. 103 (a) based upon these references are overcome and should be withdrawn.

Rejections under 35 U.S.C. 103(a) over WO 97/35991 in view of Golden et al. and Garboczi et al.

Claim 13 was rejected under 35 U.S.C. 103(a) as being unpatentable in view of WO 97/35991 in view of Golden et al. and Garboczi et al. (1996), *J. Immunol.* 157: 5403-5410 ("Garboczi et al."). Claim 13 is cancelled herein; however, Applicants have amended claim 1 and claim 11 to incorporate the limitation of claim 13. Respectfully, Applicants must disagree with grounds for the rejection.

The Office Action states that WO 97/35991 teaches "soluble (i.e. extracellular domains) recombinant divalent and multivalent analogs (including tetravalent, i.e. a tetramer) of heterodimeric proteins and pharmaceutical compositions thereof, including αβ TCR that possess enhanced affinity for their target molecules, said αβ TCRs being associated via Ig linker molecules which may further comprise a toxin, and /or may be further linked by association via avidin." The Office Action acknowledges, however, that WO 97/35991 "does not teach multivalent soluble αβ TCR wherein each chain has a heterologous C-terminal dimerization peptide which is a coiled coil domain (such as a leucine zipper from c-fos and c-jun) dimerization peptide, which dimerize, one with the other, and wherein a short flexible linker is between the TCR and the dimerization domain, and further, wherein a disulfide bond present in the native TCR between the α and β chains adjacent to the cytoplasmic domain is absent from the recombinant TCR." Thus, WO 97/35991 does not teach the present invention.

The Office Action states that the Golden et al. reference teach "soluble heterodimeric TCR comprising an α and a β chain, each chain comprising a leucine zipper which dimerizes, one with the other, produced in E.coli at yields of 4-5 mg/L. However, Applicants note that Golden et al. specifically teaches a heterodimeric TCR which forms the same intrachain disulfide bond found in native TCR. Golden et al. states on page 163 (Abstract) that the α and β chains "formed proper inter- and intrachain disulfide bonds."

Furthermore, Golden et al. teach that elimination of the disulphide bond leads to an undesirable perturbation of the structure of the TCR. Golden et al. distinguish between correctly and incorrectly folded forms of a recombinant TCR, "sD10TCR-LZ" as follows:

> "The reactivity of TCR β-chain specific (H57) and family specific (Vα2 and Vβ8.1/8.2) mAbs to purified sD10TCR-LZ was investigated by western blot analysis. Under non-reducing conditions, the ~59 kDa sD10 TCR-LZ heterodimer reacted with all mAbs (Fig. 5, lanes 1, 2, 3)...Under reducing conditions, all three antibodies failed to detect sD10TCR-LZ protein (Fig. 5, lanes 4, 5, 6), implying that recognition by these conformationally sensitive antibodies is dependent on disulfide bond formation." (Page 167, column 2, lines 1-6, and page 168, column 1, lines 3-7).

As is known in the art, nonreducing conditions preserve disulphide bonds in proteins, whereas reducing conditions break these bonds. Thus, Golden et al. teach that reducing conditions, which lead to the elimination of disulphide bonds, also lead to a loss of the conformational integrity of the proteins.

Therefore, because Golden et al. specifically teach the inclusion of the interchain disulphide bond, and because Golden et al. also teach that elimination of the interchain disulphide bond results in the loss of the properly folded forms of the TCR α and β chains, the Golden et al. reference teaches away from the present invention in which the disulphide bonds are absent.

The Office Action states that Garboczi et al. teach "a soluble TCR without the interchain disulfide bond present in native TCRs, and that the heterodimerization, refolding, and antigenic specificity of the TCR do not require its interchain disulfide bond, transmembrane segments or glycosylation (especially Abstract and page 5408, column 1)." The Office Action then suggests that "it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have a recombinant TCR as taught by the combination of WO97/35991 and Golden et al. without the disulfide bond as taught by Garboczi et al."

Respectfully, Applicants must disagree with the characterizations of Garboczi et al., and the conclusion of obviousness. In particular, Applicants submit that the Garboczi et al. reference (a) is directed to producing and storing TCR heterodimers at high concentrations and low temperatures which are not relevant to the present invention, (b) teaches a method which, in fact, leads to unstable TCR heterodimers under conditions which are relevant to the present invention, and (c) teaches the production of recombinant proteins which differ structurally from those of WO97/35991 and the present invention, and from which one cannot reasonably predict the folding properties of other proteins.

The Garboczi et al. reference is primarily directed to the production of very high concentrations of soluble TCR protein for crystallization and subsequent X-ray crystallographic analysis. Therefore, the TCR heterodimers of Garboczi et al. need be "stable" only at the high concentrations required for crystal formation, as opposed to the lower concentrations used for *in vitro* diagnostic or *in vivo* therapeutic uses. Thus, Garboczi et al. teaches that:

> "Proteins (10 ug each) were placed in 10 mL of Tris or PBS, and then mixed with 3 mL of 50% glycerol for loading gels. Samples were not heated or reduced." (Page 5404, last sentence, to page 5405, first sentence).
>
> "α (45 mg) and β (35 mg) inclusion body proteins dissolved in 8 M urea were added together to ~15 mL of 6 M guanidine-HCl... at room

> temperature (RT). A liter of refolding buffer...was...cooled to 10°C. The 15-mL guanidine-HCl solution containing α and β was diluted into the refolding buffer with vigorous stirring. The refolding solution was incubated at 10°C for 6 to 12 h, and then a second 15- mL mixture of α and β was added....After 6 to 12 additional hours, a third identical 15 mL of α and β was added and incubation at 10°C continued for 24 h...The purified protein was concentrated to 36 to 100 mg/mL..." (Page 5405, second paragraph).
>
> "The refolded noncovalently associated TCR is stable and very soluble and is routinely prepared at 100 mg/mL (2 mM). Gel filtration chromatography of the TCR after 2 mo. of storage at 4°C revealed little or no formation of aggregates." (Page 5407, second paragraph).

Thus, Garboczi et al. produced and stored their TCR heterodimers at temperatures ranging from 4°C to 10°C and provided no data regarding the stability of heterodimers at room or body temperature, or at lower concentrations. In contrast, the present inventors have produced TCR heterodimers such as those taught by Garboczi et al. and found that they are not stable under such conditions. As disclosed in the specification:

> "Attempts to co-refold extracellular fragments of TCR α and β chains, truncated so that they contained the cysteine residue which *in vivo* forms a disulphide bond, produced limited success... However, when the TCR α and β chains were truncated immediately before, that is on the N-terminal side of, the cysteine residue forming the interchain disulphide bond, analytical chromatography on a Superdex G-75 column (Pharmacia) indicated that a small fraction of protein, approximately 1-2% of the amount used in the refolding reaction, had refolded into a complex of the expected molecular size for the truncated α/β heterodimer (see also Garboczi, Utz et al. 1996 for reference to method).
>
> Because incorrect disulphide bond formation can cause irreversible misfolding of protein during *in vitro* refolding, the probabilities for this to happen were sought to be minimised by mutating a cysteine residue in the TCR β constant region which is unpaired in the cellular TCR. The cysteine residue is substituted for a serine or alanine residue... Co-refolding of TCR α and β chains, both truncated immediately before the cysteine residue which forms the interchain disulphide bond, showed a dramatic improvement in yields of heterodimer, the protein fraction of correct molecular weight typically constituting 15-30% of total protein.

> However, when these soluble TCRs were stored overnight, analysis of the protein showed that the fraction with a molecular weight corresponding to the heterodimeric TCR had split into two peaks of molecular weight corresponding to the monomeric TCR α and β chains. Similar observations were made upon dilution of the soluble TCRs, indicating that α/β chain stability was low and insufficient for analyses which would require a timespan longer than a limited number of hours of dilution of the protein. In conclusion, these methods for producing soluble TCR only generated receptor with limited stability." (Specification page 62, line 14 to page 63, line 14).

Thus, not only does the Garboczi et al. reference not teach or suggest that stable TCR heterodimers without the disulphide bond can be made and used at lower concentrations and higher temperatures, but, in fact, TCR heterodimers produced according to the teachings of Garboczi et al. are not stable at those concentrations and temperatures.

Applicants submit that one of ordinary skill in the art would not have a reasonable expectation of success in predicting the folding and heterodimerization properties of the recombinant proteins of the present invention based on Garboczi et al. or Golden et al. The recombinant proteins of Garboczi et al. lack C-terminal dimerization peptides, which influence the tertiary and quaternary structure, and the recombinant proteins of Golden et al., which include C-terminal leucine zipper peptides, do not maintain a properly folded state under conditions where disulphide bonding is absent.

Therefore, Applicants respectfully submit that one would not have been motivated to combine the teachings of WO97/35991 with Golden et al. and Garboczi et al. to produce the present invention because: (1) WO97/35991 does not teach (a) multivalent soluble TCR wherein the α and β or γ and δ chains are fused to a C-terminal dimerization peptide which is a coiled coil domain, or (b) the elimination of the disulphide bond present in the native TCR between the α and β chains; and (2) Golden et al. teaches away from the elimination of the disulphide bond from TCR α and β chains which are fused to C-terminal leucine zipper dimerization domains; and (3) the Garboczi et al. reference is

(a) directed to producing and storing TCR heterodimers at high concentration and low temperatures which are not relevant to the present invention and (b) teaches a method which, in fact, leads to unstable TCR heterodimers under conditions which are relevant to the present invention and (c) teaches the production of recombinant proteins which differ structurally from those of WO 97/35991, from those of Golden et al., and from those of the present invention, and from which one cannot reasonably predict the folding and heterodimerization properties of other proteins.

In light of the foregoing, Applicants respectfully request that the rejection under 35 U.S.C. §103(a) over WO97/35991 in view of Golden et al. and Garboczi et al. be reconsidered and withdrawn.

Rejections under 35 U.S.C. 103(a) over WO 97/35991 in view of U.S. Patent No. 5,635, 563

Claims 1, 24 and 25 were rejected under 35 U.S.C. §103(a) as being unpatentable over WO 97/35991 in view of U.S. Patent 5,635,563. Applicants note that claim 13 was not rejected under 35 U.S.C. 103(a) in view of WO 97/35991 and U.S. Patent 5,635,363, as these references do not teach a recombinant TCR lacking the disulphide bond present between chains in native TCRs.

Because Applicants have amended claim 1 to incorporate a limitation corresponding to the limitation of cancelled claim 13, and because claims 24 and 25 depend directly from claim 1, Applicants submit that the rejections of claims 1, 24 and 25 under 35 U.S.C. 103 (a) based upon these references are overcome and should be withdrawn.

Rejections under 35 U.S.C. 103(a) over WO 97/35991 in view of Ahmad et al.

Claims 1 and 19-24 were rejected under 35 U.S.C. §103(a) as being unpatentable over WO 97/35991 in view of Ahmad et al. (1993) *Cancer Res.* 53: 1484-1488 (Ahmad et al.). Applicants note that claim 13 was not rejected under 35 U.S.C. 103(a) over WO

97/35991 in view of Ahmad et al., as these references do not teach a recombinant TCR lacking the disulphide bond present between chains in native TCRs.

Because Applicants have amended claim 1 to incorporate a limitation corresponding to the limitation of cancelled claim 13, and because claims 19-25 depend either directly or indirectly from claim 1, Applicants submit that the rejections of claims 1 and claims 19-24 under 35 U.S.C. 103 (a) based upon these references are overcome and should be withdrawn.

Double Patenting Rejections

The Examiner provisionally rejected claims 1-27 under the judicially created doctrine of obviousness-type double-patenting over co-pending application 09/335,087. Applicants acknowledge the provisional rejection but will defer any substantive response until it is determined that the claims are otherwise allowable.

Applicants respectfully request reconsideration of the application in light of the amendments and remarks made herein. If the Examiner believes that a telephonic interview would expedite the allowance of the application, the Examiner is invited to contact the undersigned attorney at the number below.

Respectfully submitted,
Hale and Dorr, LLP

Hollie L. Baker
Reg. No. 31,321

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Hale and Dorr, LLP
60 State Street
Boston, MA 02109
Tel. (617) 526-6110
Fax. (617) 526-5000